



How to improve the economy of bioethanol production in Serbia

Ljiljana Mojović^{a,*}, Dušanka Pejin^b, Marica Rakin^a, Jelena Pejin^b,
Svetlana Nikolić^a, Aleksandra Djukić-Vuković^a

^a Faculty of Technology and Metallurgy, University of Belgrade, Belgrade, Serbia

^b Faculty of Technology, University of Novi Sad, Serbia

ARTICLE INFO

Article history:

Received 22 January 2012

Received in revised form

28 June 2012

Accepted 15 July 2012

Available online 13 August 2012

Keywords:

Bioethanol

Triticale

Damaged wheat

Stillage

Lactic acid

ABSTRACT

Bioethanol accounts for the majority of biofuel use worldwide, either as a fuel or a gasoline enhancer. In Serbia, the industrial production of bioethanol still relies on conventional feedstocks containing starch and sugar such as corn, wheat and molasses. In order to improve the economy of bioethanol production and to avoid the competition of the feedstock utilization for food and energy, several production approaches based on crop selection, process integration and waste utilization were considered in this paper. Particular attention was put on utilization of non conventional crops such as triticale and damaged crops not appropriate for food consumption. Potential of lignocellulosic biomass for the production of second generation ethanol in Serbia was also considered as well as the utilization of stillage as a main by-product. The investigated approaches can significantly improve the economy of bioethanol production and contribute to solve serious environmental problems.

© 2012 Elsevier Ltd. All rights reserved.

Contents

1. Introduction	6040
2. Feedstocks for bioethanol production	6041
3. Production of bioethanol in Serbia	6042
4. Possibilities of using alternative or damaged crops for bioethanol production in Serbia	6043
4.1. Utilization of damaged or wasted feedstocks	6043
4.2. Triticale as a feedstock for bioethanol production	6043
4.2.1. Comparison of the costs of bioethanol production from triticale, wheat and corn	6043
4.3. Possibilities of utilization lignocellulosic biomass for the production of second generation ethanol in Serbia	6044
5. Utilization of by-products from bioethanol production	6044
5.1. Recirculation of thin stillage	6044
5.2. Utilization of stillage for the production of animal feed	6045
5.3. Utilization of thin stillage for the production of lactic acid	6045
6. Conclusions	6045
Acknowledgments	6046
References	6046

1. Introduction

Bioethanol is currently one of the most important biofuels which is both renewable and environmentally friendly [1, 2]. It can be blended with gasoline in various proportions or used as neat alcohol

* Correspondence to: University of Belgrade, Faculty of Technology and Metallurgy, Department of Biochemical Engineering and Biotechnology, Karnegijeva 4, 11000 Belgrade, Serbia. Tel.: +381 11 3370 423; fax: +381 11 3370 387.

E-mail address: lmojovic@tmf.bg.ac.rs (L. Mojović).

in dedicated engines, taking advantage of the higher octane number and higher heat of vaporization [3]. In addition, it is also an excellent fuel for future advanced flexi-fuel hybrid vehicles. The bioethanol is an oxygenated fuel containing 35% oxygen, which reduces particulate and nitrate oxides (NO_x) emissions from combustion; it is biodegradable and contributes to sustainability [2].

The production of this fuel is increasing over the years, and has reached the level of 88.7 billion liters during the year 2011 (Fig. 1) [4]. Currently, nearly all ethanol is produced by fermentation of corn glucose in the United States, or sucrose from sugar

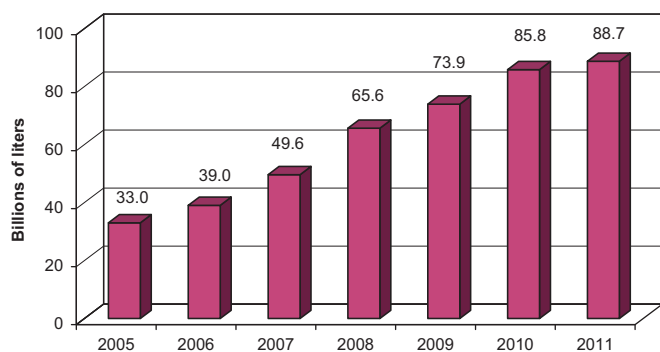


Fig. 1. World's bioethanol production in the period of 2005–2011.

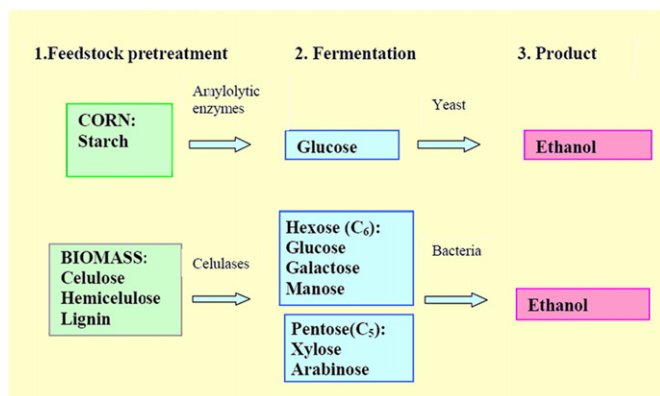


Fig. 2. Schematic presentation of bioethanol production on biomass.

cane in Brazil, but any country with a significant agronomic-based economy can use current technology for ethanol fermentation [5]. Even though ethanol production for decades mainly depended on energy crops containing starch and sugar (corn, sugar cane, etc.), new technologies for converting lignocellulosic biomass into ethanol are under development today [6].

Since bioethanol is produced by fermentation of renewable biomass, its utilization can significantly reduce fossil fuels use and exhaust greenhouse gas emission [7]. Because of that, it is expected to be one of the dominating renewable biofuels in the transportation sector within the 20 years to come [7,8]. The transportation sector itself is considered as one of the largest energy consumers as well as environmental pollutants. It accounts for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO₂) emissions [9,10]. The fact that the existing fossil fuel infrastructure, eventually with minor modification, can be used for bioethanol distribution and utilization puts this biofuel in front of other energy alternatives [8]. Consequently, an increase in bioethanol production up to more than 125 billion liters until 2020 has been predicted assuming the production support by governmental policies and exemptions [11].

Bioethanol is produced by fermentation of simple sugars present in biomass or the sugars obtained by prior chemical or enzymatic treatment of the biomass. The fermentation is performed by microorganisms, traditionally by yeasts, although some types of bacteria such as *Zymomonas mobilis* [12] could also be used. After the fermentation, the ethanol is being separated from the fermentation broth, conventionally by means of distillation and rectification or by using more efficient separation technologies such as pervaporation, membrane filtration or molecular sieves [12–14]. A schematic of bioethanol production on biomass is presented in Fig. 2.

The industrial production of bioethanol in Serbia still relies on molasses and conventional energy crops containing starch such as corn, wheat and other cereals [7,15–17]. In order to improve the economy of bioethanol production and to avoid the competition of the feedstock utilization for food and energy, several production approaches based on crop selection, process integration and waste utilization were considered: (a) utilization of damaged crops (e.g. wheat) not appropriate for food consumption; (b) utilization of triticale, the plant resistant to severe climate and soil conditions; (c) utilization of lignocellulosic biomass for the production of second generation ethanol; (d) implementation of thin stillage recirculation in the production process; (e) utilization of thin stillage for the production of lactic acid and/or microbial biomass with probiotic activity; (f) utilization of the solid stillage remained after ethanol distillation as animal feed.

2. Feedstocks for bioethanol production

Biological feedstocks that contain appreciable amounts of sugar or materials that can be converted into sugar, such as starch or cellulose can be fermented to produce bioethanol. Molasses is a traditional raw material for the production of ethanol. It is obtained as a by-product in the production of sugar from sugar beet (beet molasses) or from sugar cane (cane molasses). For a long time, molasses was practically the only raw material for the production of ethanol. However, since it can also be used for the production of other important biotechnological products such as baker's yeast, organic acids, amino acids, enzymes, etc., it is no more considered to be a cheap and widely available raw material. Currently, there is a growing interest worldwide to find out new, abundant and economically more favorable carbohydrate sources for the production of bioethanol [18–22]. A research focus is put on bioethanol production from highly abundant agricultural wastes; however, crops such as corn, wheat and sugar cane are still dominant at the industrial level.

Generally, the raw materials can be classified into three categories of agricultural raw materials: sugar-containing feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), starch materials (e.g. corn, wheat, triticale, rice, potatoes, cassava, Jerusalem artichoke, sweet potatoes and barley) and lignocellulosic materials (e.g. wood, straw and grasses).

One major problem with bioethanol production is the availability of raw materials for the production. The availability of feedstocks for bioethanol production can vary considerably from season to season and depends on geographic locations. Locally available agricultural biomass will be used for the bioethanol production [7].

Table 1
Bioethanol yields from different energy crops.

Country	Energy crop	Bioethanol yield (L/ha)
Brazil	Sugar cane, 100%	6641
USA	Corn, 98%	3770
	Sweet sorghum, 2%	1365
China	Corn, 70%	2011
	Wheat, 30%	1730
EU-27	Wheat, 48%	1702
	Sugar beet, 29%	5145
Canada	Corn, 70%	3460
	Wheat, 30%	1075
Serbia	Molasses, 50%	260 (L/t) ^a
	Cereals (wheat, corn) 50%	1700–3700

^a Bioethanol yield on molasses is given in L/t since the yield in L/ha is not applicable.

For a given production line, the comparison and choice of the feedstock includes several issues [23]: (1) chemical composition of the biomass, (2) cultivation practices, (3) availability of land and land use practices, (4) use of resources, (5) energy balance, (6) emission of greenhouse gases, acidifying gases and ozone depletion gases, (7) absorption of minerals to water and soil, (8) injection of pesticides, (9) soil erosion, (10) contribution to biodiversity and landscape value losses, (11) farm-gate price of the biomass, (12) logistic cost (transport and storage of the biomass), (13) direct economic value of the feedstock taking into account the co-products, (14) creation or maintain of employment and (15) water requirements and water availability.

Different feedstocks with various bioethanol yields are being used for bioethanol production in various world countries, as shown in Table 1 [7,10].

3. Production of bioethanol in Serbia

Current production of bioethanol in Serbia is based on molasses (50%) and cereals (50%) [12]. Today in Serbia, the bioethanol production is performed in 10 plants with a total production capacity of 40 million liters of absolute ethanol [24]. This production scale, which is now even lower than the production scale at the very end of twentieth century (see Fig. 3, presenting the bioethanol production in Serbia), is not enough to fulfill the country's ethanol needs just for beverages, medical and pharmaceutical purposes. This is a main reason why in Serbia, there is still not an organized production and utilization of bioethanol or other biofuels for gasoline substitution. Our previous analysis revealed that in Serbia, building of new bioethanol plants is necessary to produce enough bioethanol for use as a fuel in accordance with the European directive 2003/30/EC. In this context, it was estimated that about 80,000 t of bioethanol would be needed in Serbia in 2010 for 5.75% substitution of gasoline [12]. According to Directive 2009/28/EC, this amount would be close to double in 2020 and later.

Serbia is a country with a developed agricultural production. It has about 5.092 million ha of agricultural land (0.68 ha per capita), of which 4.218 million ha is arable land (0.56 ha per capita) [25]. According to available arable land per capita, it is above the European average. In the structure of the agricultural production, 70% comes from the crop field production, and 30%

from the livestock production. It is estimated that the agriculture and the industry based on agriculture (such as food and feed industry) participate in the gross domestic product (GDP) in the amount close to 40% [25].

According to current agricultural production in Serbia, starch-based raw materials for the bioethanol production are generally the most prospective. At present, close to 70% of arable land is planted with cereals, as shown in Fig. 4 [24]. The crop that is currently the most available for bioethanol production in Serbia is corn. It has been reported in 2009 that the average corn yield in Serbia was around 7 million ton, while estimated domestic needs for corn are only 4–4.5 million ton [26]. This means that there is enough corn for other purposes besides the food; therefore significant amounts can be used for the bioethanol production [26–29]. However, a growing demand for the corn and wheat on global market are currently increasing their price and make these feedstocks less appropriate. For these reasons, possibilities of using cheaper substrates such as damaged crops and triticale are being explored by the authors [7,30,31]. In addition, the

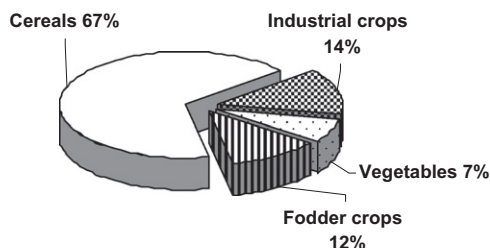


Fig. 4. Arable land planted in Serbia in 2010.

Table 2

Bioethanol yield obtained on wheat meal from hybrid Kantata from various localities at different pretreatment temperatures.

Temperature (°C)	Bioethanol yield (%)			
	Kovin	Zrenjanin	Pančevo	Vrbas
70	40.9	40.6	39.4	41.2
80	41.4	40.8	41.2	41.0
85	42.1	40.6	43.4	40.3
90	41.4	40.6	41.5	40.5

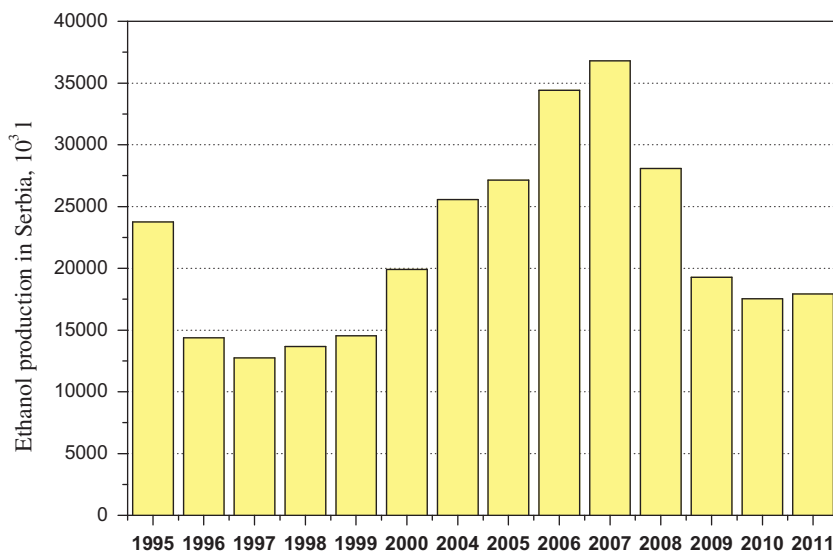


Fig. 3. Bioethanol production in Serbia in the period from 1995 to 2011.

production of bioethanol on lignocellulosic biomass should also be considered in a near future.

4. Possibilities of using alternative or damaged crops for bioethanol production in Serbia

When considering potential biomass for bioethanol production, a special attention should be paid on biomass which could be produced on marginal land. It is estimated that there are about 100,000 ha of low quality lands in Serbia which are not appropriate for conventional agricultural cultures, but could be used to cultivate alternative feedstock for bioethanol production such as sorghum, Jerusalem artichoke or triticale [32]. Another issue that should be explored is the utilization of wasted crops or that of lower quality which do not meet the food requirements. According to one global analysis [33], there are about 73.9 Tg (teragrams) of dry wasted crops in the world that could potentially produce 49.1 GL (gigaliters) of bioethanol annually. In Serbia wasted or damaged cereals could be directed into the bioethanol production.

4.1. Utilization of damaged or wasted feedstocks

Pejin et al. [34] explored the possibilities of using a domestic wheat type Kantata for the bioethanol production. This wheat type, obtained from the localities Kovin, Zrenjanin, Pančevo and Vrbas, was unsuitable for use in bakeries for bread production. However, bioethanol yields higher or close to 40% of the theoretical yields were achieved by using this feedstock. The obtained yield varied with the temperature of the pretreatment (see Table 2).

It is important to point out a positive example of economical bioethanol production on a waste substrate which is realized in a factory (Reahem, Srbobran) in the northern Serbian region of Vojvodina. The process utilizes waste water from starch processing and waste bread for the production of bioethanol. In this way, multiple benefits have been achieved, economical together with environmental.

4.2. Triticale as a feedstock for bioethanol production

Triticale (*Triticosecale*) is the intergenetic hybrid between the female parent wheat (*Triticum* spp.) and the male parent rye (*Secale* spp.). Wheat and rye belong to different genera and are well-separated by incompatibility barriers. Triticale has been transformed from a scientific curiosity to a viable crop of considerable commercial significance in the course of a few decades [35]. Recently, it has been reported that triticale is cultivated in more than 30 countries worldwide on around 3.7 million ha in total [31]. Triticale production has increased at average rate of 15,000 t/year (an approximate 18% increase per year) reaching nearly 14 million ton in 2008 [36]. Although triticale is grown in many countries of the world, the major producers are in Europe. In 2008, approximately 92% of triticale was produced in Europe, 4% in Australia, 2% Asia, 2% in America and zero in Africa [36]. Serbia could also be considered as an appropriate climate area for growing this plant.

Triticale is a crop with good yields even in less suitable conditions. It is an undemanding crop that tolerates soils with unsuitable pH and is disease and pest resistant. The straw from triticale has a heating capacity of 15.1 GJ/t at 15% moisture content [37]. Triticale also requires less agricultural chemicals (fertilizer, agronomic chemicals, etc.) due to its lower nitrogen requirement during crop growth [38,39]. Modern cultivars have been found to be very competitive as a feedstock for bioethanol production [40]. Triticale crops have a high yield potential as well

as a high starch content, together with a low content of soluble polysaccharides and protein, and is therefore considered to be ideal for bioethanol production [41]. It has been reported that triticale outyields and outperforms even the best wheat cultivars in marginal soils under unfavorable conditions, such as arid and semi-arid areas, as well as acidic soils [42]. When taking into consideration the high yield of triticale under both biotic and abiotic stress, the changing climatic conditions of the earth and its growing population, it is clear that triticale can make a contribution in future effort for sustainable bioethanol production [31].

Above and beyond the agronomic benefits triticale is perceived to have grain quality advantages that make it beneficial for fuel bioethanol production, namely higher auto-amylolytic activity than other cereals (including wheat and rye) [43]. Thus triticale has been reported as being used without any additional saccharifying enzymes [44].

In our previous research [30] it was shown that the temperature for triticale liquefaction is 35 °C which is lower than the temperature needed for corn liquefaction. Lower temperatures during the liquefaction and no need for utilization of technical enzymes, make the use of triticale as a substrate for bioethanol production more economical.

4.2.1. Comparison of the costs of bioethanol production from triticale, wheat and corn

The presented analysis of the authors of this paper compares the costs of the production of fuel bioethanol from triticale, wheat and corn. The average grain yields of triticale, wheat and corn, grown at the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad were: 9.25, 9.21, and 11.30 t/ha, respectively. Table 3 shows the data for the bioethanol yields obtained under laboratory conditions for all the investigated cereals. It can be seen that the bioethanol yields were lowest for triticale and highest for corn. These results are in agreement with the mean contents of starch of 66.67% in triticale, 68.75% in wheat, and 73.25% in corn [30].

The basis for the calculation of production costs of bioethanol from these crops were the expenses incurred in the particular phases of the production. The first production phase is the grain milling, which in the case of triticale and wheat was performed by conventional dry procedure at an energy expenditure of 31 kWh/t, whereas in the case of corn this was done using a hammer mill with corresponding energy expenditure of 46 kWh/t. The milling costs were calculated based on the average electricity price comparable with those in the EU countries (0.085 €/kWh) and they amounted 2.64 € for wheat and triticale and 3.91 € for corn. Thus, the energy consumed in the milling of triticale and wheat is by 32% lower compared to that of corn. This is in accordance with the findings of Offer and Haldenwanger [45].

A next phase in the production of bioethanol is a thermal treatment of the milled material. The corresponding costs consist of the costs of purchasing the appropriate enzymes for degradation of the starch contained in the wheat and corn samples (Table 4) and costs of energy consumed in the thermal

Table 3
Bioethanol yields from triticale, wheat and corn.

Triticale		Wheat		Corn	
Variety	Bioethanol ^a	Variety	Bioethanol ^a	Hybrid	Bioethanol ^a
NST 21/06	485.8	NS 40S	504.7	NS 640	538.0
Odisej	443.6	Rapsodija	472.7	NS 6030	528.4
Jutro	476.8	Rezensansa	483.5	NS 6010	520.0
Oganj	477.4	Dragana	519.4	NS 5043	522.7
Mean value	470.9	Mean value	495.0	Mean value	527.2

^a Liters of absolute bioethanol/tonne of dry matter.

Table 4
Calculation of the expenses of using enzymes for starch hydrolysis.

Description	Consumption norm	Triticale	Wheat	Corn
Termamyl	kg/t	0	0.20	0.50
Termamyl price	€/kg	0	5.50	5.50
Value	€	0	1.10	2.75
SAN Super 360	kg/t	0	0.60	1.00
Price of SAN Super 360 preparation	€/kg	0	7.50	7.50
Value	€	0	4.50	7.50
Total expenses	€	0	5.60	10.25

Table 5
Calculation of the energy costs of thermal treatment.

Description	Unit of measure	Triticale	Wheat	Corn
Heating temperature	°C	20–60	20–65	20–90
Energy consumed	MJ/t	563.00	633.00	1.013.00
Energy value of natural gas	MJ/m ³	33.34	33.34	33.34
Volume of consumed gas	m ³	16.89	18.99	30.39
Gas price	€/m ³	0.42	0.42	0.42
Total expenses	€	7.13	8.01	12.83

degradation of all the investigated raw materials (Table 5). The triticale varieties did not require the use of enzymes since their kernel contains amylolytic enzymes [30,43]. The amount of enzymes needed for wheat starch conversion to bioethanol was approximately by 50% lower compared to corn, because wheat kernel contains some amylolytic enzymes (Table 4). This finding is in agreement with that reported by Miedl et al. [46].

Thermal degradation in the bioethanol production is realized by heating of the mixture of milled raw material with water to a given temperature. The thermal treatment temperature depends on the nature of the raw material, and it was 60 °C for triticale, 65 °C for wheat, and 95 °C for corn [47].

From the data given in Table 5, one can see that the amount of energy needed for thermal treatment of triticale is by 44.43% smaller compared to energy for thermal treatment of corn. The thermally treated mixtures are subsequently cooled to the fermentation temperature of 30 °C and the production microorganism *Saccharomyces cerevisiae* was added in an amount of 1.25 kg/t of raw material [47].

Based on the presented results it can be concluded that triticale is advantageous for bioethanol production compared to wheat and corn. As first, in the process of bioethanol production, the milling expenses are by 32% lower compared to those for corn; there is no need for technical enzymes, and its thermal treatment requires 44% less energy compared to corn.

4.3. Possibilities of utilization lignocellulosic biomass for the production of second generation ethanol in Serbia

Currently, the starch based feedstocks are mostly utilized in Serbia for bioethanol production. However, with an exemption of wasted or damaged crops they compete with food production. For this reason, the possibilities of production of second generation bioethanol on lignocellulosic material should be considered more thoroughly in Serbia. Despite the technological problems and obstacles currently present in the production of bioethanol on lignocellulose, this feedstock is generally considered as the most promising in long turn due to its great availability and low cost, non competence with food production, as well as better environmental effects compared to the first generation of biofuels [10,48–50].

Therefore, a considerable number of pilot and demonstration plants have been announced or set up in recent years with research activities taking place mainly in North America, Europe (Denmark) and a few emerging countries (e.g. Brazil, China, India and Thailand) [51]. However, an overall price of bioethanol obtained on lignocellulose is currently still high and prevents its larger commercialization. This price is expected to decrease in the future with technological improvements, particularly with a decrease in the cost of the enzymatic pretreatment of the lignocellulosic material.

It is important to point out that Serbia is rich in biomass which could be converted into second generation ethanol. The biomass potential in Serbia represents 60% of the overall country's potential in renewable energy resources. It is estimated that the energy potential of the Serbian biomass is 2.7 Mtoe (million ton of equivalent oil) annually. The biomass consists mostly of agricultural residues (1.7 Mtoe) and forest residues (1.0 Mtoe) [12]. According to current agricultural production [24] the most available agricultural residues in Serbia are the residues of corn, wheat, sugar beet and sunflower. The authors have estimated that the amount of approximately 14 million tons of the crop residues was generated during the year 2010 which could be used for the production of second generation bioethanol (unpublished data).

According to recently accepted National action plan for biomass for 2010–2012 [52] Serbia has set a goal to substitute 2.28% of the conventional energy in transport section by biofuels 2012. The national strategy regarding the production of biofuels is still under development; however, it is clear that the efforts and investments in promoting the second generation of bioethanol in Serbia should be intensified, especially due to the fact that the feedstocks are vastly available.

5. Utilization of by-products from bioethanol production

Major by-products of bioethanol production are carbon dioxide and stillage. The carbon dioxide released during the fermentation is being collected, purified and processed in the majority of contemporary fermentation facilities. It is generally used in food industry for artificial gasification of wine and production of carbonated beverages, as well as a gas for metal processing industry [12]. An average stillage amount produced in the bioethanol process is approximately 13 hL per hL of bioethanol [53].

There are many possibilities for valorization of stillage from bioethanol processing. Some of them are the stillage recirculation and reuse [54–58], production of soil fertilizers [55], anaerobic fermentations for the production of lactic acid or butanol [59–63] and the production of various types of animal feed [64–72]. In USA, around 85% of the liquid stillage has been dried together with spent grains to produce dry distiller's grains with solubles (DDGS) which are used as animal feed. In Europe, the most of the stillage for animal feed is used in wet form because the drying itself is a costly process which requires a lot of energy [55]. In the majority of industrial facilities in Serbia, the bioethanol by-products have not been utilized, posing therefore a hardly solvable environmental problem. The complex composition of stillage causes high BOD₅ values which range from 15–340 g/L [54].

Some economical analysis have shown that the price of the by-products from bioethanol processing can reach up to 30% of the price of the primary product and thus significantly improve the production economy [12].

5.1. Recirculation of thin stillage

Thin stillage is a liquid part of the fermentation mash remaining after the distillation of bioethanol. In our previous work [54,56], a possibility of thin stillage recirculation in the mashing

process during the production of bioethanol from corn was investigated. Various process parameters such as fermentation rate, bioethanol yield, and the content of solids in stillage after distillation were evaluated. It was shown that as the amount of recirculated stillage increased (from 10% to 30%), higher bioethanol yields and starch utilization efficiencies were observed. The ethanol yield was increased from 97% to more than 100% [54], which could be explained by the fact that the stillage enriched the mashing slurry with amino acids, vitamins and the products of yeast cells degradation. The dry matter content in the slurry after the fermentation also increased with the increasing amount of the recirculated stillage. The dry matter remained after filtration of the slurry could be used as a cattle feed because of its high total protein content. This study [54] suggests that the recirculation of thin stillage should be the first and inexpensive step which should be undertaken in the bioethanol production process in order to decrease water consumption, and also the amount of the generated waste water (e.g. thin stillage). In addition, the recirculation can result in higher ethanol yield and thus improved production economy.

5.2. Utilization of stillage for the production of animal feed

Generally, the stillage obtained as a by-product from bioethanol production on cereals or various agricultural crops is a high value feed, rich in protein content and could decrease a need for the addition of other protein rich components in feed mixes. Advantages of using stillage as animal feed are following: good consumption of nutritive ingredients from the feed, good flavor of feed, presence of plant's proteins, it contains an adequate amount of energy for animals in fattening, a lot of minerals and vitamins as well as crude fibers which enhance the state of ruminant's abdomen [65,66].

During the production of bioethanol from starch feedstocks such as corn, starch is removed from the grain and converted to alcohol and carbon dioxide. As a result of starch removal, the concentration of the remaining nutrients in the grain increases approximately threefold [67]. Besides non-converted substances of the raw materials used, the stillage also contains yeast cells, the products of yeast fermentation such as the complex of B vitamins and some growth supporting compounds [64]. Due to rising feed costs, the prospect of incorporating DDGS at levels higher than traditional, or as an alternative livestock feed has become an important consideration [68,69]. Reduced starch intake increases the consumption of digestible fiber, and helps to reduce or prevent the occurrence of subacute acidosis in ruminant animals [70–72]. Besides being a good protein source in growing and finishing diets, some studies imply that corn DDGS have greater energy for growth than dry rolled corn [70,71,73].

Rakin et al. [74] analyzed chemical composition of the samples of triticale and corn stillages remained after bioethanol production, and the corn stillage enriched with 1% of *S. cerevisiae* yeast from the point of their utilization as animal feed. All stillage samples contained a high percentage of proteins (above 30%). The corn stillage enriched with 1% of *S. cerevisiae* yeast contained a maximum protein content of 42.90%. The addition of the yeast contributed to an increase in protein content, fat content and the content of particular minerals such as P, Ca and Cu. The triticale stillage contained more minerals such as Zn, Fe, Mg and Ca, compared to the corn stillage.

Nowadays, an integrated model of bioethanol production makes possible utilization of the enriched stillage for animal feeding and the manure from animal feeding for the production of biogas. A stabilized sludge remained in the process can be used as a fertilizer. Besides, by direct combustion of lignocelluloses

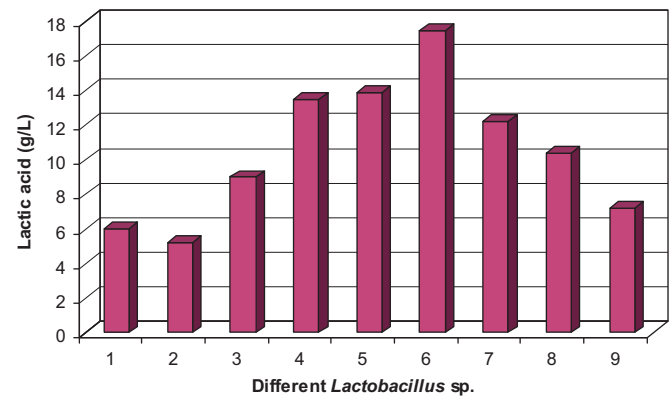


Fig. 5. Fermentative activity of different species of *Lactobacillus* in corn liquid stillage after 72 h of lactic fermentation. (1) *Lb. fermentum* NRRL B-75624, (2) *Lb. fermentum* PL-1, (3) *Lb. plantarum* PL-4, (4) *Lb. pentosus* NRRL B-227, (5) *Lb. casei* ssp. *casei* NRRL B-441, (6) *Lb. paracasei* ssp. *paracasei* NRRL B-4564, (7) *Lb. rhamnosus* ATCC 7469, (8) *Lb. acidophilus* ATCC 4356, (9) *Lb. helveticus* ATCC 15009.

part of the plants a considerably energy for heating can be released [75].

5.3. Utilization of thin stillage for the production of lactic acid

In addition to other above mentioned applications, the thin stillage from bioethanol production could be used as an inexpensive renewable feedstock for lactic acid production. This idea is particularly supported by the world growing demand for lactic acid due to its versatile and increasing utilization in pharmaceutical, food and chemical industry. Serbia itself imports lactic acid and this approach could be of great interest. There are several recent studies dealing with the utilization of corn thin stillage [60–62,76–78] and triticale stillage [59] for this purpose. Lactic acid fermentation was conducted with nine different species from *Lactobacillus* genera and their growth, sugar utilization and lactic acid production on the stillage were compared [60]. Among them, the most productive strains on corn stillage was *Lb. casei* ssp. *casei* NRRL B-441 (see Fig. 5) which produced around 17 g/L of lactic acid at 41 °C [60]. The most appropriate species for lactic acid fermentation of triticale stillage was *Lb. fermentum* PL-1 [59]. The results have shown that both of the stillages were good substrates for the growth of lactic acid bacteria (LAB) and lactic acid fermentation.

By optimization of the fermentation conditions lactic acid yield of 18.6 g/L and high biomass production (3×10^8 CFU/mL) were realized on the stillage from the production of bioethanol on waste bread by *Lb. rhamnosus* ATCC 7469 [62]. In this study possibilities of parallel production of lactic acid and probiotic biomass were assessed. Although a precise economical analysis has not been performed yet, a positive economical effect of thin stillage utilization is already obvious, as well as a positive environmental effect.

6. Conclusions

Because of the growing trend of bioethanol production and its utilization as an alternative biofuel, mostly for transportation sector, the economy of this production should be analyzed and improved in order to decrease the production costs and make this biofuel competitive with fossil fuels. In this view, the choice of suitable and abundant raw material is of great importance since the feedstock cost represents a major part of the production cost. Our analysis has compared the cost of bioethanol produced from three crops that can be cultivated in Serbia: corn, wheat and triticale, and the triticale has been shown as the most favorable.

Utilization of waste crops or by-products from other industries such as starch processing, could also be a very rational approach. The efforts and investments in promoting the second generation of bioethanol on lignocellulosic biomass in Serbia should be intensified, especially due to the fact that this biomass is vastly available in the country and it doesn't compete with food production. In addition, a proper utilization of by-products from bioethanol production for the production of animal feed and lactic acid can significantly improve the economy of bioethanol production.

Acknowledgments

This work was funded by the Serbian Ministry of Education and Science (TR 31017).

References

- [1] Balat M, Balat H. Progress in bioethanol processing. *Progress in Energy and Combustion Science* 2008;34:551–73.
- [2] Balat M, Balat H. Recent trends in global production and utilization of bioethanol fuel. *Applied Energy* 2009;86:2273–82.
- [3] Kim S, Dale BE. Environmental aspects of ethanol derived from no-tilled corn grain: nonrenewable energy consumption and greenhouse gas emissions. *Biomass Bioenergy* 2005;28:475–89.
- [4] Global Renewable Fuels Alliance. <http://www.globalrfa.org/pr_021111.php> [accessed September 2011].
- [5] Semenčenko V, Mojović L, Đukić-Vuković A, Radosavljević M, Terzić M, Milašinović Šeremešić M. Suitability of some selected maize hybrids from Serbia for bioethanol and dried distillers' grains with solubles (DDGS) production. *Journal of the Science of Food and Agriculture*. <http://dx.doi.org/10.1002/jsfa.5801>, in press.
- [6] Semenčenko V, Mojović L, Petrović S, Očić O. Recent trends in bioethanol production. *Hemijaska industrija* 2011;65:103–14.
- [7] Mojović L, Pejčin D, Grujić O, Markov S, Pejčin J, Rakin M, et al. Progress in the production of bioethanol on starch-based feedstocks. *Chemical Industry and Chemical Engineering Quarterly* 2009;15:221–6.
- [8] International Energy Agency (IEA). Key world energy statistics, Paris, OECD/IEA; 2008.
- [9] Goldemberg J. Environmental and ecological dimensions of biofuels. In: *Proceedings of the conference on the ecological dimensions of biofuels*; Washington DC; 2008. p.12.
- [10] Balat M. Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. *Energy Conversion and Management* 2011;52:858–75.
- [11] Demirbas A. Producing and using bioethanol as an automotive fuel. *Energy Sources Part B* 2007;2:391–401.
- [12] Mojović L, Pejčin D, Lazić M. Bioethanol kao gorivo – stanje i perspektive. Leskovac: Tehnološki fakultet; 2007.
- [13] Zhou L, Wang T, Nguyen QT, Long J, Li Y, Ping Z. Cordierite-supported ZSM-5 membrane: preparation and pervaporation properties in the dehydration of water–alcohol mixture. *Separation and Purification Technology* 2005;44:266–70.
- [14] Koczka K, Mizsey P, Fonyo Z. Rigorous modelling and optimization of hybrid separation processes based on pervaporation. *Central European Journal of Chemistry* 2007;5(4):1124–47.
- [15] Dodić S, Popov S, Dodić J, Ranković J, Zavargo Z. Potential development of bioethanol production in Vojvodina. *Renewable & Sustainable Energy Review* 2009;13:2722–7.
- [16] Dodić S, Vučurović V, Popov S, Dodić J, Zavargo Z. Concept of cleaner production in Vojvodina. *Renewable & Sustainable Energy Review* 2010;14:1629–34.
- [17] Dodić S, Popov S, Dodić J, Ranković J, Zavargo Z, Jevtić-Mučibabić R. Bioethanol production from raw juice as intermediate of sugar beet processing. *Biomass Bioenergy* 2009;33:822–7.
- [18] Mohanty SM, Behera S, Swain MR, Ray RC. Bioethanol production from mahula (*Madhuca latifolia* L.) flowers by solid state fermentation. *Applied Energy* 2009;86:640–4.
- [19] Demirbas MF, Balat M, Balat H. Biowastes-to-biofuels. *Energy Conversion and Management* 2011;52:1815–28.
- [20] Velmurugan R, Muthukumar K. Sono-assisted enzymatic saccharification of sugarcane bagasse for bioethanol production. *Biochemical Engineering Journal* 2012;63:1–9.
- [21] Romão B, Da Silva F, De Resende M, Cardoso V. Ethanol production from hydrolyzed soybean molasses. *Energy and Fuels* 2012;26:2310–6.
- [22] Li S, Zhang X, Andresen JM. Production of fermentable sugars from enzymatic hydrolysis of pretreated municipal solid waste after autoclave process. *Fuel* 2012;92:84–8.
- [23] Gnansounou E, Bedniaguine D, Dauriat A. Promoting bioethanol production through clean development mechanism: findings and lessons learnt from ASIATIC project. In: *Proceedings of the 7th IAAE European energy conference*, Bergen, Norway; August 28–30, 2005.
- [24] Statistical Office of the Republic of Serbia. <<http://webzrzs.stat.gov.rs/WebSite/>> [accessed September 2011].
- [25] Chamber of Commerce and Industry of Serbia. <<http://www.pks.rs/PrivredaSrbije.aspx?id=13&p=28>> [accessed June 2012].
- [26] Nikolić S, Mojović L, Rakin M, Pejčin D. Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of *Saccharomyces cerevisiae* var. *ellipsoideus*. *Fuel* 2009;88:1602–7.
- [27] Nikolić S, Mojović L, Pejčin D, Rakin M, Vučurović V. Improvement of ethanol fermentation of hydrolyzates of corn semolina by immobilized *Saccharomyces cerevisiae* var. *ellipsoideus* by media supplementation. *Food Technology & Biotechnology* 2009;47:83–9.
- [28] Mojović L, Nikolić S, Rakin M, Vukašinović M. Production of bioethanol from corn meal hydrolyzates. *Fuel* 2006;85:1750–5.
- [29] Nikolić S, Mojović L, Rakin M, Pejčin D, Savić D. A microwave-assisted liquefaction as a pretreatment for bioethanol production by the simultaneous saccharification and fermentation of corn meal. *Chemical Industry and Chemical Engineering Quarterly* 2008;14:231–4.
- [30] Pejčin D, Mojović L, Vučurović V, Pejčin J, Denčić S, Rakin M. Fermentation of wheat and triticale hydrolyzates: a comparative study. *Fuel* 2009;88:1625–8.
- [31] Pejčin D, Mojović L, Pejčin J, Grujić O, Markov S, Nikolić S, et al. Increase in bioethanol production yield from triticale by simultaneous saccharification and fermentation with application of ultrasound. *Journal of Chemical Technology & Biotechnology* 2012;87:170–6.
- [32] Baras J, Gačeša S, Pejčin D. Ethanol is a strategic raw material. *Hemijaska industrija* 2002;56:89–105.
- [33] Kim S, Dale BE. Global potential bioethanol production from wasted crops and crop residues. *Biomass Bioenergy* 2004;26:361–75.
- [34] Pejčin D, Vučurović V, Popov S, Dodić J, Dodić S. Production of ethanol from. *Kantata* wheat variety. *Acta Periodica Technologica* 2006;37:155–61.
- [35] Oettler G. The fortune of a botanical curiosity – triticale: past, present and future. *Journal of Agricultural Science* 2005;143:329–46.
- [36] Food and Agriculture Organization of the United Nations, FAO; 2010 <<http://faostat.fao.org/>> [accessed May 2011].
- [37] Havířková K, Suchý J. Development model for energy crop plantations in the Czech Republic for the years 2008–2030. *Renewable & Sustainable Energy Review* 2010;14:1925–35.
- [38] Čiftić I, Yenice E, Eleroglu H. Use of triticale alone and in combination with wheat or maize: effects of diet type and enzyme supplementation on hen performance, egg quality, organ weights, intestinal viscosity and digestive system characteristics. *Animal Feed Science and Technology* 2003;105:149–61.
- [39] Davis-Knight H, Weightman R. The potential of triticale as a low input cereal for bioethanol production. The Home-Grown Cereals Authority (HGCA) project report No 434. ADAS UK Ltd, Centre for Sustainable Crop Management, Cambridge, UK; 2008.
- [40] Eudeus F. Canadian triticale biorefinery initiative. In: *Proceedings of the 6th international triticale symposium*, Stellenbosch, South Africa; 2006. p. 85–88.
- [41] Boros D. Triticale of high end-use quality enhances opportunities to increase its value in world cereal market. In: *Proceedings of the 6th international triticale symposium*, Stellenbosch, South Africa; 2006. p. 119–125.
- [42] Tohver M, Kann A, Täht R, Mihhavelski A, Hakman J. Quality of triticale cultivars suitable for growing and bread-making in northern conditions. *Food Chemistry* 2005;89:125–32.
- [43] Kučerova J. The effect of year, site and variety on the quality characteristics and bioethanol yield of winter triticale. *Journal of the Institute of Brewing* 2007;33:142–6.
- [44] Senn T, Pieper H. Classical methods. In: Roehr M, editor. *The biotechnology of ethanol-classical and future applications*. Weinheim, Germany: Wiley-Vch Verlag GmbH; 2001. p. 7–86.
- [45] Offer G, Haldenwanger M. Zerkleinerungsgrad der Rohstoffe beim drucklosen Stärkeaufschluss als Voraussetzung für einen optimalen Brennereibetrieb. *Branntweinwirtschaft* 1998;128:178–85.
- [46] Miedl M, Cornfine S, Leiper K, Shepher M, Stewart G. Low-temperature processing of wheat for bioethanol production, part I. Studies on the use of commercial enzymes. *Journal of the American Society of Brewing Chemists* 2007;65:183–91.
- [47] Ingledew WM. Alcohol production by *Saccharomyces cerevisiae*: a yeast primer. In: Jacques KA, Lyons TP, Kelsall DR, editors. *The alcohol textbook*. Nottingham, United Kingdom: Nottingham University Press; 1999. p. 49–52.
- [48] Joshi B, Bhatt MR, Sharma D, Joshi J, Malla R, Sreerama L. Lignocellulosic ethanol production: current practices and recent developments. *Biotechnology and Molecular Biology Reviews* 2011;6:172–82.
- [49] Margeot A, Hahn-Hagerdal B, Edlund M, Slade R, Mono F. New improvements for lignocellulosic ethanol. *Current Opinion in Biotechnology* 2009;20:372–80.
- [50] Li X, Mupondwa E, Panigrahi S, Tabil L, Sokhansanj S, Stumborg M. A review of agricultural crop residue supply in Canada for cellulosic ethanol production. *Renewable & Sustainable Energy Review* 2012;16:2954–65.
- [51] Sustainable Production of Second-Generation Biofuels, © OECD/IEA 2010 <http://www.progressivefuelslimited.com/PDF/second_generation_biofuels.pdf> [accessed June 2011].

- [52] Republika Srbija, Akcioni plan za biomasu 2010–2012, <<http://www.ssl-link.com/mre/cms/mestoZaUploadFajlove/BAPsrpski.pdf>> [accessed May 2011].
- [53] Kim JS, Kim BG, Lee CH, Kim SW, Jee Koh JH, Fan AG. Development of clean technology in alcohol fermentation industry. *Journal of Cleaner Production* 1997;5:263–7.
- [54] Pejlin D, Mojović L, Grujić O, Pejlin J, Rakin M. The bioethanol production with the thin stillage recirculation. *Chemical Industry and Chemical Engineering Quarterly* 2009;15:49–52.
- [55] Banković-Ilić I, Lazić M, Veljković V, Mojović L, A stillage – secondary product of ethanol fermentation. In: *Proceedings of the 7th symposium of new technologies and economical development*, Leskovac, Serbia, CD edition; October 19–20, 2007. p. 100–107.
- [56] Đukić-Vuković A, Nikolić S, Mojović L, Pejlin D, Rakin M, Pejlin J, et al. The possibilities of utilization of stillage from the production of bioethanol on starch feedstocks. In: D. Chiaramonti and V.N. Raina (Eds.), *Proceedings of XIX ISAF international symposium of alcohol fuels*, Verona; October 10–14, 2011. p. 415–420.
- [57] Morin-Couallier E, Fargues C, Lewandowski R, Decloux M, Lameloise ML. Reducing water consumption in beet distilleries by recycling the condensates to the fermentation phase. *Journal of Cleaner Production* 2008;16:655–63.
- [58] Gumienna M, Lasik M, Szambelan K, Czarnecki Z. Reduction of water consumption in bioethanol production from triticale by recycling the stillage liquid phase. *Acta Scientiarum Polonorum. Technologia Alimentaria* 2011;10: 467–74.
- [59] Marković M, Markov S, Pejlin D, Mojović L, Vukašinović M, Pejlin J, et al. The possibility of lactic acid fermentation in the triticale stillage. *Chemical Industry and Chemical Engineering Quarterly* 2011;17:153–62.
- [60] Mojović L, Vukašinović Sekulić M, Đukić A, Pejlin D, Rakin M, Pejlin J, et al. Production of lactic acid on liquid distillery stillage. *Journal on Processing and Energy in Agriculture* 2011;15:1–5.
- [61] Đukić-Vuković A, Mojović L, Vukašinović-Sekulić M, Rakin M, Nikolić S, et al. Utilization of the stillage from bioethanol production on waste bread for lactic acid and biomass production. *Journal on Processing and Energy in Agriculture* 2012;16:11–6.
- [62] Djukić-Vuković A, Mojović L, Vukašinović-Sekulić M, Rakin M, Nikolić S, et al. Effect of different fermentation parameters on L-lactic acid production from liquid distillery stillage. *Food Chemistry* 2012;134:1038–43.
- [63] Krzywonos M, Cibis E, Miśkiewicz T, Ryznar-Luty A. Utilization and biodegradation of starch stillage (distillery wastewater). *Electronic Journal of Biotechnology* 2009;12. <<http://www.ejbiotechnology.info/content/vol12/issue1/full/5/index.html>>.
- [64] Mojović L, Pejlin D, Rakin M, Vukašinović M, Pejlin J, Grujić O, et al. Investigation of possibilities of utilization of the stillage from bioethanol production. *Journal on Processing and Energy in Agriculture* 2010;14:54–7.
- [65] Lan Y, Opapeju FO, Nyachoti CM. True ileal protein and amino acid digestibilities in wheat dried distillers' grains with solubles fed to finishing pigs. *Animal Feed Science and Technology* 2008;140:155–63.
- [66] Robinso P, Karges K, Gibson ML. Nutrition evaluation of four co-product feedstuffs from the motor fuel ethanol distillation industry in midwestern USA. *Animal Feed Science and Technology* 2008;146:345–52.
- [67] Spiehs M, Whitney M, Shurson G. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. *Journal of Animal Science* 2002;80:2639–45.
- [68] Beckman J, Keeney R, Tyner W. Feed Demands and Coproduct Substitution in the Biofuel era. *Agribusiness* 2011;27:1–18.
- [69] Au F, McKeown L, McAllister TA, Chaves AV. Fermentation characteristics of corn-, triticale-, and wheat-based dried distillers grains with solubles in barley-based diets determined using continuous and batch culture systems. *Journal of the Science of Food and Agriculture* 2010;90:2074–82.
- [70] Ham G, Stock R, Klopfenstein T, Larson E, Shain D, Huffman R. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. *Journal of Animal Science* 1994;72:3246–57.
- [71] Klopfenstein T. Distillers grains as an energy source and effect of drying on protein availability. *Animal Feed Science and Technology* 1997;60:201–7.
- [72] Klopfenstein T, Erickson G, Bremer V. Use of distillers byproducts in the beef cattle feeding industry. *Journal of Animal Science* 2008;86:1223–31.
- [73] Nuez Ortin WG, Yu P. Nutrient variation and availability of what DDGS, corn DDGS and blend DDGS from bioethanol plants. *Journal of the Science of Food and Agriculture* 2009;89:1754–61.
- [74] Rakin M, Mojović L, Nikolić S, Vukašinović-Sekulić M, Pejlin D. Enhancement of spent grain quality for animal feed after bioethanol production. *Ecologica* 2009;16:151–4.
- [75] Pejlin D, Glavardanov D, Gačeša S, Popov S. Alcohol as a fuel – future trends. In: *Book of papers of the 5th congress of the industry of alcohol, nonalcoholic beverages and vinegar*, Vrnjačka Banja, Serbia; 2000. p. 29–38.
- [76] Đukić Vuković A, Mojović L, Pejlin D, Vukašinović-Sekulić M, Rakin M, Nikolić S, et al. New trends and challenges in lactic acid production on renewable biomass. *Hemijaska industrija* 2011;65:411–22.
- [77] Đukić-Vuković A, Rakin M, Mojović L, Vukašinović-Sekulić M, Nikolić S. Possibilities to lactic acid production on different agricultural by-products. In: *8th European congress of chemical engineering/1st European congress of applied biotechnology (ECCE/ECAB)*; 2011, P36.23.
- [78] Zhang L, Shi K, Shi G. Production of L-lactic acid with very high gravity distillery wastewater from ethanol fermentation by a newly isolated *Enterococcus hawaiiensis*. *Journal of Chemical Technology & Biotechnology* 2011;86:213–6.